

PATENT COOPERATION TREATY

PCT

REC'D 30 MAR 2001
WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT (PCT Article 36 and Rule 70)

Applicant's or agent's file reference 80135/WO	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/EP00/01797	International filing date (<i>day/month/year</i>) 02/03/2000	Priority date (<i>day/month/year</i>) 11/03/1999
International Patent Classification (IPC) or national classification and IPC A61K35/74		
<p>Applicant SOCIETE DES PRODUITS NESTLE S.A. et al</p> <p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 9 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 2 sheets.</p> <p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> I <input checked="" type="checkbox"/> Basis of the report II <input checked="" type="checkbox"/> Priority III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input checked="" type="checkbox"/> Certain documents cited VII <input checked="" type="checkbox"/> Certain defects in the international application VIII <input checked="" type="checkbox"/> Certain observations on the international application 		
Date of submission of the demand 22/09/2000	Date of completion of this report 28.03.2001	
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Fayos, C Telephone No. +49 89 2399 2180	



INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/01797

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*):
Description, pages:

1-19 as originally filed

Claims, No.:

1-10 with telefax of 28/02/2001

Drawings, sheets:

1/5-5/5 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- the language of publication of the international application (under Rule 48.3(b)).
- the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- contained in the international application in written form.
- filed together with the international application in computer readable form.
- furnished subsequently to this Authority in written form.
- furnished subsequently to this Authority in computer readable form.
- The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- the description, pages:
- the claims, Nos.:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/01797

- the drawings, sheets:
5. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)): *(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*
6. Additional observations, if necessary:

II. Priority

1. This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
- copy of the earlier application whose priority has been claimed.
- translation of the earlier application whose priority has been claimed.
2. This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
see separate sheet

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 5
	No:	Claims 1-4 and 6-10
Inventive step (IS)	Yes:	Claims -
	No:	Claims 1-10
Industrial applicability (IA)	Yes:	Claims 1-10
	No:	Claims -

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/EP00/01797

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP00/01797

Re Item II

Priority

- 1- The priority date (11.03.1999) of the present application is valid.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

- 2- Reference is made to the following documents:

- D1: EP-A-0 577 903 (NESTLE SA) 12 January 1994 (1994-01-12) cited in the application
- D2: WO 98 06411 A (DICOFARM SPA ;GUANDALINI STEFANO (IT)) 19 February 1998 (1998-02-19)
- D3: US-A-5 837 238 (MOLLSTAM BO ET AL) 17 November 1998 (1998-11-17)
- D4: KAILA M ET AL: 'Viable Versus Inactivated Lactobacillus Strain GG in Acute Rotavirus Diarrhoea' ARCHIVES OF DISEASE IN CHILDHOOD, vol. 72, no. 1, 1 January 1995 (1995-01-01), pages 51-53, XP002058045
- D5: EP-A-0 861 905 (PROGE FARM S R L) 2 September 1998 (1998-09-02)
- D6: WO 95 33046 A (BRACCO SPA ;DIBRA SPA (IT); MORELLI LORENZO (IT); BOTTAZZI VITTORI) 7 December 1995 (1995-12-07)
- D7: TUOMOLA E.M. ET AL: 'Adhesion of some probiotic and dairy Lactobacillus strains to Caco-2 cell cultures' INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY, vol. 41, 1998, pages 45-51, XP002115137
- D8: see item VI below
- D9: DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US PRASAD, JAYA ET AL: 'Selection and characterisation of Lactobacillus and Bifidobacterium strains for use as probiotics.' retrieved from STN XP002121832 & INTERNATIONAL DAIRY JOURNAL, (1998) VOL. 8, NO. 12, PP. 993-1002.,
- D10: MIETTINEN, MINJA (1) ET AL: 'Lactobacilli and streptococci induce interleukin-12 (IL-12), IL-18, and gamma interferon production in human peripheral blood

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP00/01797

mononuclear cells.' INFECTION AND IMMUNITY, (DEC., 1998) VOL. 66, NO. 12, PP. 6058-6062., XP002121831

D11: MAJAMAA H ET AL: 'LACTIC ACID BACTERIA IN THE TREATMENT OF ACUTE ROTAVIRUS GASTROENTERITIS' JOURNAL OF PEDIATRIC GASTROENTEROLOGY AND NUTRITION, US, RAVEN PRESS, NEW YORK, NY, vol. 20, no. 3, 1 April 1995 (1995-04-01), pages 333-338, XP002058047 ISSN: 0277-2116

NOVELTY - Art. 33 (1) and (2) PCT

3- Claims 1-5 and 7-13 are not novel for the following reasons:

- Note: - Claims 1-4 are product claims. A product (here a Lactic acid bacterium strain belonging to the genus *Lactobacillus*) is only defined by its components / elements and not by its intended use ("preventing colonization of the intestine with pathogenic bacteria causing diarrhoea and preventing infection of intestinal epithelial cells by rotaviruses"). Lactic acid bacterium strains belonging to the genus *Lactobacillus* are well known (see below), in particular *Lactobacillus rhamnosus* or *Lactobacillus paracasei*.
- Claims 5-7 and 9-10 merely refer to the preparation of an ingestible support material containing the product of any of claims 1-6 (well known - see below).
- Claim 8 refers to the use of a preparation according to any of claims 7-10 for the treatment and / or prophylaxis of disorders associated with diarrhoea (well known - see below).
- "capable to grow in the presence of up to 0,4 % bile salts" is considered a parameter, by which a Lactic acid bacterium strain belonging to the genus *Lactobacillus* can be characterized, in this case.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP00/01797

- 3.4- D5 discloses the use of Lactobacilli (belonging to the species Paracasei and Salivarius) capable of adhering to the cells of the intestinal mucosa and stable to bile juices (see p 4 lines 4-5 - 0,5% bile salts) for the manufacture of compositions (10^6 - $3 \cdot 10^9$ c.f.u. per unitary dose) such as milk and yogurt for the treatment of e. g. diarrhoea (p 4 line 24 - p 6 line 35).

Therefore, in the light of D5, claims 1-3 and 5-10 are not novel.

- 3.7- Lactobacillus paracasei CNCM I-2116 (NCC 2461) appears to be formally novel over the cited prior art, since it is not strictly disclosed in any of the available prior art documents. Hence, claim 4 can be considered as being novel over the cited prior art.

INVENTIVE STEP - Art. 33 (1) and (3) PCT

- 4- Claims 1-3 and 5-10, which are not novel, cannot be considered as being inventive, and claim 4 lacks inventive step for the reasons stated below.**

- 4.1- The closest prior art is represented by D5.

The closest prior art differs from the present application in that it does not explicitly mention Lactobacillus paracasei CNCM I-2116 (NCC2461)

The technical effect achieved in the present application is the prevention of colonization of the intestine with pathogenic bacteria causing diarrhoea and the prevention of infection of intestinal epithelial cells by rotaviruses, using Lactobacillus paracasei CNCM I-2116 (NCC 2461).

The objective problem posed in the present application is to provide additional bacterial strains that exhibit properties beneficial for man and / or animals.

The solution proposed is Lactobacillus paracasei CNCM I-2116 (NCC 2461).

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP00/01797

Lactic acid bacterium strains belonging to the genus *Lactobacillus* such as *Lactobacillus rhamnosus* or *Lactobacillus paracasei* are known and have been used for therapeutical purposes.

- 4.2- The *Lactobacilli* of D5 have the following characteristics: stability to acids, stability to bile juices, capability of adhering to cells of the intestinal mucosa, and capability of producing only (or anyway in high prevalence) L(+) lactic acid (p 4-5). Furthermore, it is to be noted that *Lactobacilli* in general are catalase negative and gram positive and are either homofermentative (produce lactic acid) or heterofermentative (produce ethyl alcohol, CO₂, and often other acids in addition to lactic acid).

The *lactobacilli* of D5 are also isolated from human faeces (see e. g. p 3 line 23) and share typical structural and functional characteristics with the *Lactobacillus paracasei* strain of the present application, i. e. CNCM I-2116.

Since the present application does not provide any technical data concerning the isolation and characterization of *L. paracasei* CNCM I-2116 (see examples), which would differentiate it from the *lactobacilli* identified in D5, there is no reason to believe that *L. paracasei* CNCM I-2116 is functionally different from the strains of D5.

- 4.3- The subject matter of claims 1-10 is therefore obvious in the light of D5.

- 4.4- Further note that, although none of D1-D4, D6-D7 and D11 explicitly mention that the Lactic acid bacteria belonging to the genus *Lactobacillus* they disclose are capable to grow in the presence of up to 0,4% bile salts, it is obvious that, when aiming at providing a bacterial preparation having a positive effect in the intestine of a subject, the skilled man would only have selected those strains which are capable of growing in the presence of up to 0,4% bile salts. Hence, any of D1-D4, D6-D7 and D11, alone or in combination with D5 also anticipate the subject matter of the present application.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP00/01797

Re Item VI

Certain documents cited

- 5- Certain published documents (Rule 70.10): D8

Application No Patent No	Publication date (day/month/year)	Filing date (day/month/year)	Priority date (<i>valid claim</i>) (day/month/year)
WO 99/29833	17.06.99	08.12.98	08.12.97

Re Item VII

Certain defects in the international application

- 6- The relevant prior art should be identified in the description (Rule 5.1(1)(a)(ii) PCT).
- 7- It is not clear from the description of the present application that ST11 corresponds to the *L. casei* strain CNCM I-2116, since the specification therefor first appears on p 6 § 3 (Art. 5 PCT).

Re Item VIII

Certain observations on the international application

- 8- There appears to be an inconsistency between the claims (e. g. claims 4-6) and the description (e. g. p 6 § 3-4 among others) since the claims refer to *Lactobacillus paracasei* and the description refers to *lactobacillus casei*.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 80135/WO	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/EP 00/01797	International filing date (<i>day/month/year</i>) 02/03/2000	(Earliest) Priority Date (<i>day/month/year</i>) 11/03/1999
Applicant SOCIETE DES PRODUITS NESTLE S.A. et al		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
 - the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :
 - contained in the international application in written form.
 - filed together with the international application in computer readable form.
 - furnished subsequently to this Authority in written form.
 - furnished subsequently to this Authority in computer readable form.
 - the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
 - the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. Certain claims were found unsearchable (See Box I).

3. Unity of invention is lacking (see Box II).

4. With regard to the title,

- the text is approved as submitted by the applicant.
- the text has been established by this Authority to read as follows:

LACTIC ACID BACTERIA STRAINS CAPABLE OF PREVENTING DIARRHOEA

5. With regard to the abstract,

- the text is approved as submitted by the applicant.
- the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

- as suggested by the applicant.
- because the applicant failed to suggest a figure.
- because this figure better characterizes the invention.

None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/01797

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K35/74 C12N1/20 A23L1/29 A23C9/152 // (C12N1/20, C12R1:225)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 577 903 A (NESTLE SA) 12 January 1994 (1994-01-12) cited in the application the whole document ---	1,6-11
X	WO 98 06411 A (DICOFARM SPA ; GUANDALINI STEFANO (IT)) 19 February 1998 (1998-02-19) the whole document ---	1-11
X	US 5 837 238 A (MOLLSTAM BO ET AL) 17 November 1998 (1998-11-17) the whole document ---	1,2,6-11

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

5 June 2000

13/06/2000

Name and mailing address of the ISA

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Fax: (+31-70) 340-3016

Authorized officer

Fernandez y Branas, F

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/01797

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KAILA M ET AL: "Viable Versus Inactivated Lactobacillus strain GG in Acute Rotavirus Diarrhoea" Archives of Disease in Childhood, vol. 72, no. 1, 1 January 1995 (1995-01-01), pages 51-53, XP002058045 the whole document ---	1-4,6-11
X	MAJAMAA H ET AL: "Lactic Acid Bacteria in the Treatment of Acute Rotavirus Gastroenteritis" Journal of Pediatric Gastroenterology and Nutrition, vol. 20, no. 3, 1 April 1995 (1995-04-01), pages 333-338, XP002058047 ISSN: 0277-2116 the whole document ---	1-4,6-11
X	EP 0 861 905 A (PROGE FARM S R L) 2 September 1998 (1998-09-02) page 4, line 25 -page 5, line 35 page 6, line 5 - line 10 ---	1-11
X	WO 95 33046 A (BRACCO SPA ;DIBRA SPA (IT); MORELLI LORENZO (IT); BOTTAZZI VITTORI) 7 December 1995 (1995-12-07) page 9 -page 12 page 13 -page 27 ---	1-4,6-11
X	TUOMOLA E.M. ET AL: "Adhesion of some probiotic and dairy Lactobacillus strains to Caco-2 cell cultures" INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY, vol. 41, 1998, pages 45-51, XP002115137 the whole document ---	1-11
X,P	WO 99 29833 A (FONDEN RANGNE ;SVENSSON ULLA (SE); ALEJUNG PER (SE); ARLA EKONOMI) 17 June 1999 (1999-06-17) the whole document ---	1-4,6-11
X	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US PRASAD, JAYA ET AL: "Selection and characterisation of Lactobacillus and Bifidobacterium strains for use as probiotics." retrieved from STN XP002121806 abstract & INTERNATIONAL DAIRY JOURNAL, (1998) VOL. 8, NO. 12, PP. 993-1002., ---	1-3

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/01797

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MIETTINEN, MINJA (1) ET AL: "Lactobacilli and streptococci induce interleukin-12 (IL-12), IL-18, and gamma interferon production in human peripheral blood mononuclear cells." INFECTIO AND IMMUNITY, (DEC., 1998) VOL. 66, NO. 12, PP. 6058-6062., XP002121805 the whole document -----	1-11

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 00/01797

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP 0577903	A 12-01-1994	AT 161181 T AU 4158893 A CA 2099855 A CZ 9301342 A DE 69223615 D DE 69223615 T DK 577903 T ES 2111625 T FI 933001 A GR 3025971 T HU 66632 A JP 2672247 B JP 6098782 A NO 932407 A NZ 248056 A PL 299543 A SK 71193 A US 5578302 A		15-01-1998 13-01-1994 07-01-1994 19-01-1994 29-01-1998 09-04-1998 06-04-1998 16-03-1998 07-01-1994 30-04-1998 28-12-1994 05-11-1997 12-04-1994 07-01-1994 28-08-1995 21-02-1994 08-06-1994 26-11-1996
WO 9806411	A 19-02-1998	IT RM960571 A AU 3863997 A EP 0918531 A		09-02-1998 06-03-1998 02-06-1999
US 5837238	A 17-11-1998	AU 3154897 A EP 0923293 A PL 330324 A WO 9746104 A		05-01-1998 23-06-1999 10-05-1999 11-12-1997
EP 0861905	A 02-09-1998	IT MI970426 A		27-08-1998
WO 9533046	A 07-12-1995	IT 1269838 B IT 1274733 B AT 163042 T AU 686389 B AU 2613995 A BR 9507752 A DE 69501591 D DE 69501591 T DE 760848 T EP 0760848 A ES 2099056 T FI 964681 A GR 97300016 T GR 3026758 T HU 75555 A JP 10500577 T NO 964966 A NZ 287420 A US 5709857 A		15-04-1997 24-07-1997 15-02-1998 05-02-1998 21-12-1995 07-10-1997 12-03-1998 10-06-1998 28-08-1997 12-03-1997 16-05-1997 08-01-1997 31-05-1997 31-07-1998 28-05-1997 20-01-1998 23-01-1997 19-12-1997 20-01-1998
WO 9929833	A 17-06-1999	SE 510813 C AU 1896899 A SE 9704577 A		28-06-1999 28-06-1999 09-06-1999

PATENT COOPERATION TREATY

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NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents
 United States Patent and Trademark
 Office
 Box PCT
 Washington, D.C.20231
 ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 19 October 2000 (19.10.00)

International application No. PCT/EP00/01797	Applicant's or agent's file reference 80135/WO
International filing date (day/month/year) 02 March 2000 (02.03.00)	Priority date (day/month/year) 11 March 1999 (11.03.99)

Applicant RENIERO, Roberto et al

1. The designated Office is hereby notified of its election made:

in the demand filed with the International Preliminary Examining Authority on:

22 September 2000 (22.09.00)

in a notice effecting later election filed with the International Bureau on:

2. The election was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Juan Cruz Telephone No.: (41-22) 338.83.38
---------------------------------------------------------------------------------------------------------------------------------------	-------------------------------------------------------------------------

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

4. März 2001

To:

STRAUS, Alexander
BECKER-KURIG-STRAUS
Bavariastrasse 7
D-80336 München
ALLEMAGNE

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL PRELIMINARY
EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing
(day/month/year) 28.03.2001

Applicant's or agent's file reference
80135/WO

IMPORTANT NOTIFICATION

International application No. PCT/EP00/01797	International filing date (day/month/year) 02/03/2000	Priority date (day/month/year) 11/03/1999
-------------------------------------------------	----------------------------------------------------------	----------------------------------------------

Applicant
SOCIETE DES PRODUITS NESTLE S.A. et al

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/

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Authorized officer

Hundt, D

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 80135/WO	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP00/01797	International filing date (day/month/year) 02/03/2000	Priority date (day/month/year) 11/03/1999
International Patent Classification (IPC) or national classification and IPC A61K35/74		
Applicant SOCIETE DES PRODUITS NESTLE S.A. et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

- I Basis of the report
- II Priority
- III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV Lack of unity of invention
- V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI Certain documents cited
- VII Certain defects in the international application
- VIII Certain observations on the international application

Date of submission of the demand 22/09/2000	Date of completion of this report 28.03.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Fayos, C Telephone No. +49 89 2399 2180



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/EP00/01797

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).*):

Description, pages:

1-19 as originally filed

Claims, No.:

1-10 with telefax of 28/02/2001

Drawings, sheets:

1/5-5/5 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- the language of publication of the international application (under Rule 48.3(b)).
- the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- contained in the international application in written form.
- filed together with the international application in computer readable form.
- furnished subsequently to this Authority in written form.
- furnished subsequently to this Authority in computer readable form.
- The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- the description, pages:
- the claims, Nos.:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/EP00/01797

the drawings, sheets:

5. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

II. Priority

1. This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

- copy of the earlier application whose priority has been claimed.
 translation of the earlier application whose priority has been claimed.

2. This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
see separate sheet

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 5
	No:	Claims 1-4 and 6-10
Inventive step (IS)	Yes:	Claims -
	No:	Claims 1-10
Industrial applicability (IA)	Yes:	Claims 1-10
	No:	Claims -

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/EP00/01797

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item II

Priority

- 1- The priority date (11.03.1999) of the present application is valid.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

- 2- Reference is made to the following documents:

- D1: EP-A-0 577 903 (NESTLE SA) 12 January 1994 (1994-01-12) cited in the application
- D2: WO 98 06411 A (DICOFARM SPA ;GUANDALINI STEFANO (IT)) 19 February 1998 (1998-02-19)
- D3: US-A-5 837 238 (MOLLSTAM BO ET AL) 17 November 1998 (1998-11-17)
- D4: KAILA M ET AL: 'Viable versus inactivated Lactobacillus strain GG in acute rotavirus diarrhoea' ARCHIVES OF DISEASE IN CHILDHOOD, vol. 72, no. 1, 1 January 1995 (1995-01-01), pages 51-53, XP002058045
- D5: EP-A-0 861 905 (PROGE FARM S R L) 2 September 1998 (1998-09-02)
- D6: WO 95 33046 A (BRACCO SPA ;DIBRA SPA (IT); MORELLI LORENZO (IT); BOTTAZZI VITTORI) 7 December 1995 (1995-12-07)
- D7: TUOMOLA E.M. ET AL: 'Adhesion of some probiotic and dairy Lactobacillus strains to Caco-2 cell cultures' INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY, vol. 41, 1998, pages 45-51, XP002115137
- D8: see item VI below
- D9: DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US PRASAD, JAYA ET AL: 'Selection and characterisation of Lactobacillus and Bifidobacterium strains for use as probiotics.' retrieved from STN XP002121832 & INTERNATIONAL DAIRY JOURNAL, (1998) VOL. 8, NO. 12, PP. 993-1002.,
- D10: MIETTINEN, MINJA (1) ET AL: 'Lactobacilli and streptococci induce interleukin-12 (IL-12), IL-18, and gamma interferon production in human peripheral blood

mononuclear cells.' INFECTION AND IMMUNITY, (DEC., 1998) VOL. 66, NO. 12, PP. 6058-6062., XP002121831

D11: MAJAMAA H ET AL: 'LACTIC ACID BACTERIA IN THE TREATMENT OF ACUTE ROTAVIRUS GASTROENTERITIS' JOURNAL OF PEDIATRIC GASTROENTEROLOGY AND NUTRITION, US, RAVEN PRESS, NEW YORK, NY, vol. 20, no. 3, 1 April 1995 (1995-04-01), pages 333-338, XP002058047 ISSN: 0277-2116

NOVELTY - Art. 33 (1) and (2) PCT

3- Claims 1-5 and 7-13 are not novel for the following reasons:

Note:

- Claims 1-4 are product claims. A product (here a Lactic acid bacterium strain belonging to the genus *Lactobacillus*) is only defined by its components / elements and not by its intended use ("preventing colonization of the intestine with pathogenic bacteria causing diarrhoea and preventing infection of intestinal epithelial cells by rotaviruses"). Lactic acid bacterium strains belonging to the genus *Lactobacillus* are well known (see below), in particular *Lactobacillus rhamnosus* or *Lactobacillus paracasei*.
- Claims 5-7 and 9-10 merely refer to the preparation of an ingestible support material containing the product of any of claims 1-6 (well known - see below).
- Claim 8 refers to the use of a preparation according to any of claims 7-10 for the treatment and / or prophylaxis of disorders associated with diarrhoea (well known - see below).
- "capable to grow in the presence of up to 0,4 % bile salts" is considered a parameter, by which a Lactic acid bacterium strain belonging to the genus *Lactobacillus* can be characterized, in this case.

- 3.4- D5 discloses the use of Lactobacilli (belonging to the species Paracasei and Salivarius) capable of adhering to the cells of the intestinal mucosa and stable to bile juices (see p 4 lines 4-5 - 0,5% bile salts) for the manufacture of compositions (10^6 - 3.10^9 c.f.u. per unitary dose) such as milk and yogurt for the treatment of e. g. diarrhoea (p 4 line 24 - p 6 line 35).

Therefore, in the light of D5, claims 1-3 and 5-10 are not novel.

- 3.7- Lactobacillus paracasei CNCM I-2116 (NCC 2461) appears to be formally novel over the cited prior art, since it is not strictly disclosed in any of the available prior art documents. Hence, claim 4 can be considered as being novel over the cited prior art.

INVENTIVE STEP - Art. 33 (1) and (3) PCT

- 4- Claims 1-3 and 5-10, which are not novel, cannot be considered as being inventive, and claim 4 lacks inventive step for the reasons stated below.**

- 4.1- The closest prior art is represented by D5.

The closest prior art differs from the present application in that it does not explicitly mention Lactobacillus paracasei CNCM I-2116 (NCC2461)

The technical effect achieved in the present application is the prevention of colonization of the intestine with pathogenic bacteria causing diarrhoea and the prevention of infection of intestinal epithelial cells by rotaviruses, using Lactobacillus paracasei CNCM I-2116 (NCC 2461).

The objective problem posed in the present application is to provide additional bacterial strains that exhibit properties beneficial for man and / or animals.

The solution proposed is Lactobacillus paracasei CNCM I-2116 (NCC 2461).

Lactic acid bacterium strains belonging to the genus Lactobacillus such as *Lactobacillus rhamnosus* or *Lactobacillus paracasei* are known and have been used for therapeutical purposes.

- 4.2- The Lactobacilli of D5 have the following characteristics: stability to acids, stability to bile juices, capability of adhering to cells of the intestinal mucosa, and capability of producing only (or anyway in high prevalence) L(+) lactic acid (p 4-5). Furthermore, it is to be noted that Lactobacilli in general are catalase negative and gram positive and are either homofermentative (produce lactic acid) or heterofermentative (produce ethyl alcohol, CO₂, and often other acids in addition to lactic acid).

The lactobacilli of D5 are also isolated from human faeces (see e. g. p 3 line 23) and share typical structural and functional characteristics with the *Lactobacillus paracasei* strain of the present application, i. e. CNCM I-2116.

Since the present application does not provide any technical data concerning the isolation and characterization of *L. paracasei* CNCM I-2116 (see examples), which would differentiate it from the lactobacilli identified in D5, there is no reason to believe that *L. paracasei* CNCM I-2116 is functionally different from the strains of D5.

- 4.3- The subject matter of claims 1-10 is therefore obvious in the light of D5.

- 4.4- Further note that, although none of D1-D4, D6-D7 and D11 explicitly mention that the Lactic acid bacteria belonging to the genus Lactobacillus they disclose are capable to grow in the presence of up to 0,4% bile salts, it is obvious that, when aiming at providing a bacterial preparation having a positive effect in the intestine of a subject, the skilled man would only have selected those strains which are capable of growing in the presence of up to 0,4% bile salts. Hence, any of D1-D4, D6-D7 and D11, alone or in combination with D5 also anticipate the subject matter of the present application.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP00/01797

Re Item VI

Certain documents cited

- 5- Certain published documents (Rule 70.10): D8

Application No Patent No	Publication date (day/month/year)	Filing date (day/month/year)	Priority date (valid claim) (day/month/year)
WO 99/29833	17.06.99	08.12.98	08.12.97

Re Item VII

Certain defects in the international application

- 6- The relevant prior art should be identified in the description (Rule 5.1(1)(a)(ii) PCT).
- 7- It is not clear from the description of the present application that ST11 corresponds to the L. casei strain CNCM I-2116, since the specification therefor first appears on p 6 § 3 (Art. 5 PCT).

Re Item VIII

Certain observations on the international application

- 8- There appears to be an inconsistency between the claims (e. g. claims 4-6) and the description (e. g. p 6 § 3-4 among others) since the claims refer to Lactobacillus paracasei and the description refers to lactobacillus casei.

Claims

1. Lactic acid bacterium belonging to the genus Lactobacillus capable of adhering to and essentially colonizing the intestinal mucosa, capable of preventing infection of 5 intestinal epithelial cells by rotaviruses and capable to grow in the presence of up to 0.4 % bile salts.
2. The Lactobacillus according to claim 1, which is selected from the group consisting of Lactobacillus rhamnosus or Lactobacillus paracasei.
10
3. The Lactobacillus according to claim 2, which is Lactobacillus paracasei.
4. The Lactobacillus paracasei strain according to claim 3, which is Lactobacillus paracasei CNCM I-2116.
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5. Use of a lactic acid bacterium according to any of the preceding claims for the preparation of a ingestable support material.
6. The use according to claim 5, wherein the Lactobacillus strain is contained in 20 the support material in an amount from about 10^5 cfu / g to about 10^{12} cfu / g support material.
7. The use according to any of the claims 5 or 6, wherein the support material is a food composition selected from milk, yogurt, curd, cheese, fermented milks, milk 25 based fermented products, ice-creams, fermented cereal based products, milk based powders, infant formulae.
8. The use according to any of the claims 5 to 6, wherein the support material is used for the treatment and/or prophylaxis of disorders associated with diarrhea.

9. Food or pharmaceutical composition containing at least one lactic acid bacterium according to any of the claims 1 to 3.
- 5 10. The composition according to claim 9, which is selected from milk, yogurt, curd, cheese, fermented milks, milk based fermented products, ice-creams, fermented cereal based products, milk based powders, infant formulae, tablets, liquid bacterial suspensions, dried oral supplement, wet oral supplement, dry tube feeding or wet tubefeeding.

10

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